AD NUMBER

AD843595

NEW LIMITATION CHANGE

TO

Approved for public release, distribution unlimited

FROM

Distribution authorized to U.S. Gov't. agencies and their contractors; Administrative/Operational Use; SEP 1968. Other requests shall be referred to the Army Biological Laboratory, Attn: Technical Release Branch [TID], Fort Detrick, MD 21702.

AUTHORITY

BDRL, per d/a ltr dtd 29 Sep 1971

AD

AD843595

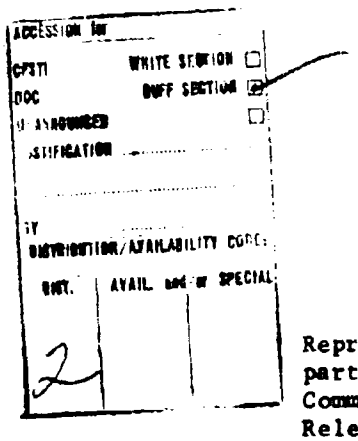
TECHNICAL MANUSCRIPT 474

IMMUNOFLUORESCENT CELL-COUNTING ASSAY OF RIFT VALLEY FEVER VIRUS

Nicholas Hahon

SEPTEMBER 1968

DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland



DDC AVAILABILITY NOTICES

Qualified requesters may obtain copies of this publication from DDC.

Foreign announcement and dissemination of this publication by DDC is not authorized.

Release or announcement to the public is not authorized.

DISPOSITION INSTRUCTIONS

Destroy this publication when it is no longer needed. Do not return it to the originator.

The findings in this publication are not to be construed as an official Department of the Army position, unless so designated by other authorized documents.

DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland 21701

TECHNICAL MANUSCRIPT 474

IMMUNOFLUORESCENT CELL-COUNTING ASSAY OF RIFT VALLEY FEVER VIRUS

Nicholas Hahon

Experimental Aerobiology Division
AEROBIOLOGY AND EVALUATION LABORATORY

Project 1B522301A059

September 1968

In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the Committee on the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Sciences-National Research Council.

ACKNOWLEDGMENT

The technical assistance of W. Douglas Zimmerman is gratefully appreciated.

ABSTRACT

Rift Valley fever virus was quantitatively assayed by enumeration of cells containing fluorescent viral antigens after infection of L cell monolayers. The efficiency and speed of virus attachment were markedly enhanced when augmented by centrifugal force. Approximately 97% of virus inoculum attached to cells within 10 minutes. Thus, a proportionality was obtained between the number of infected cells and volume of inoculum. Virus penetration into cells was linear and complete within 30 minutes at 35 C. From observations on the sequential development of viral antigen within cells and fluorescent cell counts, infected cells were enumerated as early as 12 hours after inoculation of cell monolayers. A linear function was demonstrated between infected cell counts and virus concentration. The immunofluorescent cell-counting assay of Rift Valley fever virus was more precise, sensitive, and rapid than assays based on intraperitoneal inoculation of mice or cytopathic effects in cell cultures.

CONTENTS

FIGURES

TABLES

I. INTRODUCTION

General agreement does not exist on the most suitable host system for assay of Rift Valley fever (RVF) virus as regards maximal sensitivity or minimal variability. Although mice, hamsters, embryonated chicken eggs, and tissue cultures have been commonly employed,[1] independent evaluations of various assay systems by different investigators have often given conflicting or equivocal results.[2-8]

Recently, quantitative assays were developed for two arboviruses, yellow fever[9] and Venezuelan equine encephalomyelitis (VEE),[10] based on immunofluorescent staining of infected cell monolayers and enumeration of cells containing fluorescent viral antigens. These assays are direct (each infected cell is the result of infection by one viable virus particle) and dependent on a primary cycle of infection. Some outstanding features of these assays are the relatively short time required to obtain estimates of virus infectivity (less than 24 hours), high sensitivity, and precision.

The feasibility of extending the immunofluorescent cell-counting technique to assess RVF virus infectivity was investigated because cells infected with the virus are amenable to immunofluorescent staining.[4,11] This report describes the development and evaluation of this technique for the quantitative assay of RVF virus.

II. MATERIALS AND METHODS

A. VIRUS

The van Wyk strain of pantropic RVF virus, originally isolated in the Union of South Africa from sheep blood,[12] was used throughout this study. A virus suspension in the form of infectious lamb serum was obtained from Dr. Peter Gerone, Fort Detrick. It had a titer of $1 \times 10^{7.6}$ mouse intraperitoneal LD_{50} per ml.

B. CELL LINES AND CULTIVATION

The L-929 strain of mouse fibroblasts was routinely employed in the assay of RVF virus. Nutrient medium for the cells consisted of medium 199 containing 10% fetal calf serum (FCS) and 50 µg of streptomycin per ml and 75 µg of kanamycin per ml. Cells were maintained in medium 199 and 5% FCS. Other established cell lines employed in the assay were McCoy, hamster

kidney, and guinea pig lung. Growth medium for McCoy cells was medium 199, 10% FCS, and 0.5% (w/v) lactalbumin hydrolysate (LAH); for hamster kidney cells, medium 199 and 10% FCS; and for guinea pig lung, basal medium Eagle (BME) with 10% FCS and 0.5% LAH. Maintenance medium for hamster kidney and McCoy cell cultures was the same as that used for L cells; BME and 5% FCS was used for guinea pig lung cells.

For virus assay, cells were cultivated on circular cover slips (15-mm diameter) inserted in flat-bottomed glass vials (19 by 65 mm). A 1-ml amount of cell suspension, containing from 1 x 10^5 to 3 x 10^5 cells, was introduced onto cover slips that were then incubated at 35 C for 24 hours, or until a complete cell monolayer was formed.

C. VIRUS ASSAY

Determinations were usually carried out in triplicate. Virus dilutions were prepared in phosphate-buffered saline (PBS), pH 7.1, free of calcium and magnesium ions. This consisted of 8.5 g of NaCl, 1.07 g of Na_2HPO_4 (anhydrous), and 0.39 g of $NaH_2PO_4 \cdot 2H_2O$ per liter of distilled water. Inoculum in 0.2-ml volume was introduced directly onto cover slip cell monolayers after their transfer from glass vials to rotor chamber inserts.[9] The latter were employed because they withstand the high centrifugal force required to sediment the virus. Rotor chamber inserts placed in a swinging-bucket SW 25.1 rotor were centrifuged in a model L Preparative Ultracentrifuge* at 19,642 to 29,432 x g for 15 minutes at 30 C. The residual inoculum was removed after centrifugation, the cover slip cell monolayers were replaced into glass vials, and 1 ml of maintenance medium was then added to each vial. After incubation at 35 C for 1 hour, the maintenance medium was replaced with 1 ml of a 1/30 dilution of virus antiserum in medium 199. The rationale for this procedure is presented in Section III. After further incubation at 35 C for 20 to 22 hours, cover slip cell monolayers were rinsed twice with cold PBS, fixed with cold (-60 C) acetone, and either prepared immediately for immunofluorescent staining and cell counting or stored at -60 C. Fluorescence of viral antigens in fixed cell cultures was not diminished when they were stored under these conditions for 7 weeks.

The median dose of virus producing a cytopathic effect in L cell monolayers maintained in serum-free medium[8] and the method of intraperitoneal inoculation of Swiss-Webster strain mice, 10 to 14 g,[13] were also used to assay virus.

D. RVF ANTISERUM CONJUGATE AND IMMUNOFLUORESCENT STAINING

RVF antiserum was obtained from rhesus monkeys that had been aerogenically vaccinated 1 month earlier. Antiserum was conjugated with fluorescein isothiocyanate by the method of Riggs et al.[14] The conjugated globulin was

* Beckman Instruments, Inc., Spinco Division, Palo Alto, Calif.

passed through a Sephadex G-50 column to remove unbound dye. To reduce nonspecific fluorescence, 5 ml of conjugated globulin were diluted with an equal volume of PBS and adsorbed twice with 200 mg of acetone-dried mouse liver powder in accord with the procedure of Coons and Kaplan.[16]

The direct fluorescent antibody method was employed to demonstrate immunofluorescence of viral antigens in infected cells. Fixed cell cultures were washed once with PBS and stained with conjugated antiserum for 30 minutes at room temperature. Cover slip cell monolayers were then rinsed in two changes of PBS and mounted in a semipermanent medium.[18]

E. FLUORESCENCE MICROSCOPY AND CELL COUNTING

Cover slip cell cultures were examined with an American Optical microscope equipped with a Fluorolume illuminator (Model 645), Corning No. 5840 and Schott BG-12 exciter filters, and an E.K. No. 2A barrier filter. With this optical system at a magnification of 430X, the number of microscopic fields contained in the area of a 15-mm cover slip was 1,064. For each cover slip cell monolayer, 50 microscopic fields were examined for fluorescent cells. To calculate the number of cell-infecting units (CIU) of virus per milliliter, the average number of fluorescent cells per field was multiplied by the number of fields per cover slip, the reciprocal of the dilution of virus inoculum, and a volume factor (for conversion to milliliters).

F. DETERMINATION OF VIRUS ATTACHMENT

Attachment was measured by following the disappearance of virus from inoculum after its addition to cell monolayers. Cover slip monolayers were inoculated with 0.2 ml of a 10^{-5} virus dilution. After designated intervals of incubation or centrifugation, residual inoculum was removed, and the cell cultures were immediately washed twice with PBS. Residual inoculum was then introduced onto fresh cell monolayers to measure unattached virus. For this, the residual inoculum was adsorbed onto cell cultures by centrifugation at 19,642 to 29,432 x g for 30 minutes. Cover slip cell monolayers exposed to initial or residual inocula were then treated in the manner described earlier for virus assay. The amount of virus that was attached to cells at a given time was expressed as a percentage of the virus input. The input was the sum of the amounts of attached and free virus.

G. DETERMINATION OF VIRUS PENETRATION

Virus penetration into cells was measured by the insensitivity of attached virus to antiserum. Inoculum was attached to cell monolayers with the aid of centrifugal force. Cell cultures were washed twice with PBS, overlayed at designated intervals with 1 ml of a 1/50 dilution of virus antiserum, and then incubated at 35 C for 20 to 22 hours. The quantity of virus that penetrated cells at a given time was expressed as a percentage of the input virus.

H. CALCULATION OF ATTACHMENT AND PENETRATION CONSTANTS

The attachment and penetration rate constants were calculated from the relationship, 2.3 log $(V_0/V_t)/nt$, where V_0 = the input virus concentration, V_t = unattached or unpenetrated virus at time t, and n = the number of cells per cm^3 determined by resuspension of cover slip cell cultures and enumeration.

III. RESULTS

A. VIRUS ATTACHMENT

The rate of RVF virus attachment onto cover slip L cell monolayers was determined for both centrifugation and stationary incubation. Virus inputs for the former and the latter were 4,149 and 8,469 CFU, respectively. The concentration of cells per cover slip culture was 5.3×10^5 per ml. Additional experimental details are described in Section II.

The rate of virus attachment with each treatment is shown in Figure 1. Aided by centrifugal force, 97% of the virus inoculum was attached within 10 minutes; after stationary incubation for 2 hours, only 30% of the virus inoculum was attached. The attachment rate constant was 2.4×10^{-7} cm^3 min^{-1} with centrifugation and 5.1×10^{-9} cm^3 min^{-1} with stationary incubation. These values are markedly similar to those reported for the attachment of VEE virus under similar experimental conditions.[10] The findings demonstrate the efficiency and rapidity of virus attachment to cells when augmented by centrifugal force.

The efficiency of centrifugation for infecting cell monolayers from different volumes of inoculum is shown in Table 1. The results show a proportionality between the number of infected RVF virus cells and volume of inoculum. A comparable study with yellow fever virus demonstrated a similar relationship.[9]

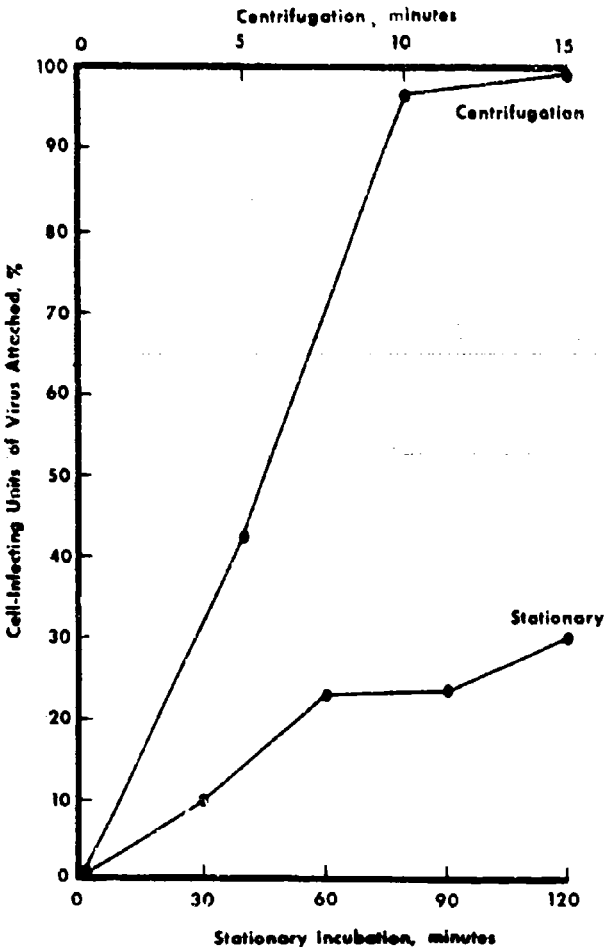


Figure 1. Attachment of RVF Virus onto Cover Slip Cultures of L Cells by Centrifugation and Stationary Incubation.

TABLE 1. PROPORTIONALITY BETWEEN VOLUME OF INOCULUM AND CELL-INFECTION UNITS OF RVF VIRUS

Volume, ml	Infected Cells/ 50 Fields[a]	CIU/ml x 10^9
0.1	59	2.5
0.2	114	2.4
0.5	305	2.5
1.0	615	2.6

a. Mean of two determinations.

Studies carried out on the primary interactions between VEE virus and cell cultures revealed that virus attachment was markedly influenced by the menstruum used to suspend the virus. Maximal attachment of VEE virus occurred only in the presence of a specific monovalent cation.[10] To determine whether the attachment of RVF virus to cell monolayers was also highly dependent on the composition of the diluent, serial dilutions of virus were made in six different suspending media, pH 7.1 to 7.3. A 1/300,000 dilution of virus in each test medium was the inoculum. Virus attachment was carried out with the aid of centrifugation. Residual inoculum was then removed and replaced with maintenance medium, and the cell monolayers were incubated and treated in accord with the prescribed assay procedure.

Results in Table 2 show that the composition of the medium employed for the attachment of RVF virus to cell monolayers did not significantly affect assay values. Heart infusion broth was the exception; the quantity of virus attached was approximately 50% of the amount attained with the other media. In general, the attachment requirement for RVF virus to cells was less specific than that encountered with VEE virus.[10]

TABLE 2. EFFECT OF ATTACHMENT MEDIUM ON ASSAY OF RVF VIRUS

Attachment Medium	Infected Cells/100 Fields		CIU/ml x 10^9
	Test 1	Test 2	
PBS[a]	77	72	1.2
Medium 199 + 5% FCS	63	-[b]	1.0
Medium 199 + 10% FCS	68	76	1.1
Medium 199 + 20% FCS	68	88	1.2
PBS + 0.0009 M $CaCl_2$ and 0.0005 M $MgCl_2$	80	79	1.3
Heart infusion broth	42	33	0.6

a. NaCl (0.15 M) buffered by 0.01 M phosphate.
b. - = Not done.

B. VIRUS PENETRATION

The rate of virus penetration into cells was followed by determining the insensitivity of attached virus to antiserum at designated time intervals. A virus input of 4.8×10^3 CIU was introduced onto cell monolayers; they were then treated in the manner described in Section II. Results in Figure 2 show that virus penetration at 35 C proceeded at a linear rate after an initial lag period of almost 10 minutes. Approximately 50% of attached virus penetrated into cells within 20 minutes; the process was complete within 30 minutes. The penetration rate constant of 6.7×10^{-8} cm^3 min^{-1} for RVF virus was comparable to that reported for VEE virus.[10]

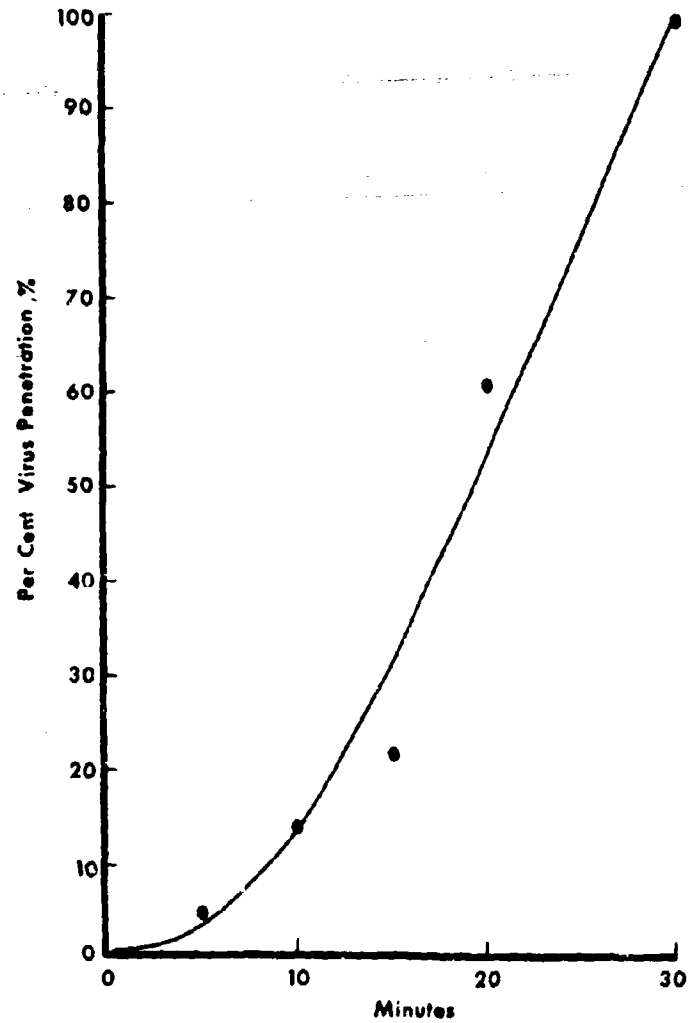


Figure 2. Rate of Penetration of RVF Virus into L Cells at 35 C as Measured by Insensitivity of Attached Virus to Antiserum.

It was important to define the penetration rate of RVF virus because preliminary observations revealed the appearance of fluorescent foci between 12 and 16 hours after infection of cell monolayers. To provide a more convenient time for enumerating individual infected cells, the addition of a virus antiserum overlay of the cell monolayers was required to prevent the initiation of a second cycle of cell infection by extracellular virus. The earliest time that the antiserum overlay may be added without neutralizing attached virus was determined from the preceding experiment. For routine assay of virus, the antiserum overlay was added after cell monolayers were incubated at 35 C for 1 hour following virus attachment.

C. INCUBATION PERIOD

The incubation period, defined here as the interval between virus inoculation and the development of recognizable quantities of viral antigen in infected cells, was established from sequential observations and counts of infected cells. The earliest visual sign of cellular infection by virus was noted at 5 hours and was in the form of minute fluorescent particles on the periphery of the nucleus. At 10 hours, the particles were distributed in large numbers throughout the cell cytoplasm and the intensity of fluorescence was markedly increased. By 12 hours, the fluorescent particles had coalesced so that infected cells could be easily discerned (Fig. 3). Fluorescent viral antigen was confined to the cell cytoplasm. Between 12 and 16 hours of incubation, fluorescent foci appeared that precluded counting of individual infected cells (Fig. 4). These foci contained five or more fluorescent cells. Fluorescent foci increased in size as the incubation time was extended. The addition of an antiserum overlay 1 hour after virus attachment to cells prevented the appearance of the foci. Counts of infected cells made at 12 hours from cell monolayers that had not received antiserum overlay were comparable to those made at 16, 20, 22, and 24 hours in the presence of the antiserum overlay. From these observations on the time of appearance of fluorescent viral antigen in cells and counts of infected cells, individual infected cells may be enumerated as early as 12 hours after virus inoculation of cell monolayers. If the incubation period is extended beyond 12 hours, an antiserum overlay must be employed to confine the infectious process to individual cells.

D. QUANTITATIVE EVALUATION OF ASSAY

A linear relationship between twofold dilutions of virus and the number of fluorescent cells is demonstrated in Figure 5. That each fluorescent cell resulted from infection by a single infective virus particle or aggregates not divisible by dilution is suggested by the data.

Figure 3. Fluorescent RVF Viral Antigen in L Cells 12 Hours after Infection. 126X.

Figure 4. L Cell Monolayer with Fluorescent Foci after Infection with RVF Virus 16 Hours Earlier. 126X.

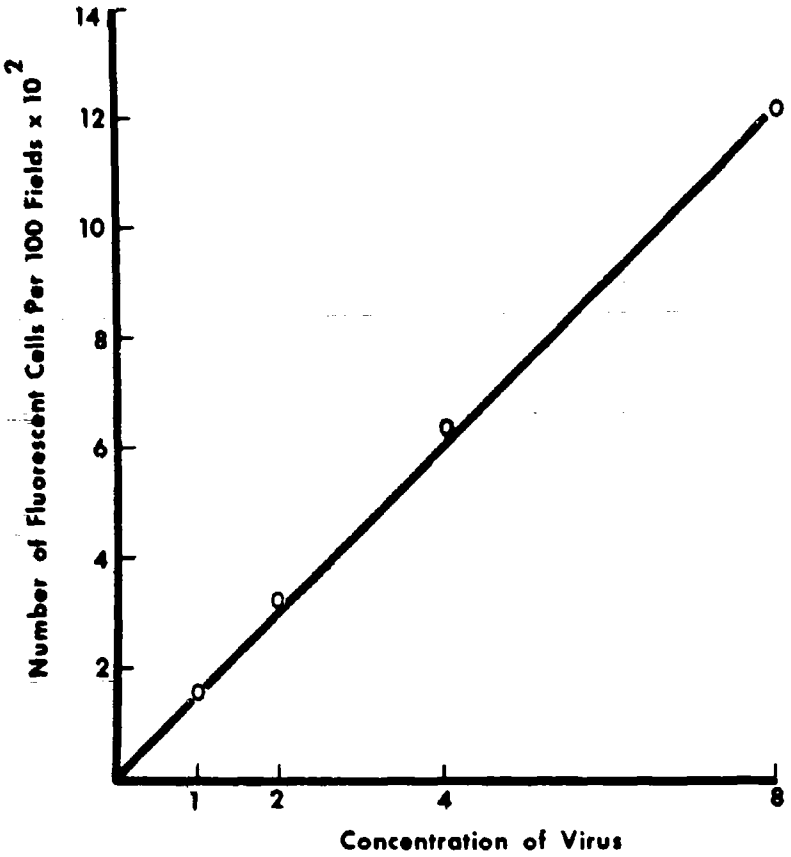


Figure 5. Linear Function Between the Number of Fluorescent Cells and Concentration of RVF Virus.

Ten determinations were performed in a single experiment to estimate the precision of the assay. Cover slip cell monolayers were infected with 0.2 ml of a 10^{-5} virus dilution and then treated in the prescribed manner. The number of CIU per ml of virus inoculum ranged from 1.6×10^9 to 2.3×10^9 with a mean of 2.0×10^9, standard deviation (SD) of ±0.27, and coefficient of variation of 13%.

Easterday and Murphy[13] demonstrated the capability of various established cell cultures to support the growth of RVF virus; however, the cell system most suitable for virus assay was not determined. To evaluate the sensitivity of different cell lines for assay of RVF virus by the fluorescent cell-counting technique, parallel dilutions of virus were introduced onto cultures of L, hamster kidney, McCoy, and guinea pig lung cells. Results of two replicate assays shown in Table 3 indicate that L cells exhibited the greatest and hamster kidney the least sensitivity to infection with RVF virus of the four cell lines tested. In L cells, virus titers were approximately 50-fold higher than in hamster kidney cells, 10-fold higher than in McCoy cells, and fourfold higher than in guinea pig lung cells.

TABLE 3. ASSAY OF RVF VIRUS IN DIFFERENT CELL LINES

Assay No.	Cell-Infecting Units of Virus per ml			
	L Cells	Guinea Pig Lung Cells	McCoy Cells	Hamster Kidney Cells
1	1.5×10^9	6.9×10^8	2.2×10^8	2.9×10^7
2	2.2×10^9	2.1×10^8	2.1×10^8	4.2×10^7
Mean	1.8×10^9	4.5×10^8	2.1×10^8	3.5×10^7

The sensitivity of the fluorescent cell-counting assay of RVF virus was compared with that of methods based on the median virus dose producing a cytopathic effect ($CPED_{50}$) and the mouse intraperitoneal median lethal dose ($MIPLD_{50}$). Results in Table 4 reveal that the fluorescent cell-counting technique was superior to that of the other two assay systems; assay values were approximately 1.7 to 2.0 log units higher and exhibited less variability.

TABLE 4. COMPARISON OF DIFFERENT METHODS FOR ASSAY OF RVF VIRUS

Assay No.	CIU[a]/ml	$CPED_{50}$[b]/ml	$MIPLD_{50}$[c]/ml
1	9.3	7.6	7.0
2	9.3	7.7	7.0
3	9.3	8.0	7.5
4	9.2	7.6	7.2
5	9.3	7.3	7.4
6	9.4	7.7	7.5
Mean	9.3	7.6	7.2
SD	±0.07	±0.23	±0.24
SE of mean	±0.02	±0.09	±0.09

a. Reciprocal of cell-infecting units of virus (log_{10}) determined by fluorescent cell-counting technique in 20 hours.
b. Reciprocal of median dose of virus (log_{10}) producing cytopathic effects in L cell monolayers in 6 days.
c. Reciprocal of median lethal mouse intraperitoneal units of virus (log_{10}) with an 8-day observation period.

IV. DISCUSSION

In the course of developing the immunofluorescent cell-counting assay of RVF virus, studies on the attachment of virus to cell monolayers indicated that the efficiency and speed of the process were significantly enhanced by centrifugal force. Approximately 97% of the virus inoculum was attached to cells within 10 minutes with the aid of centrifugal force; only 30% was attached at 2 hours with stationary incubation. These findings were similar to those reported for the attachment of two other arboviruses, yellow fever[9] and VEE.[10] When centrifugal force was employed to attach virus from different volumes of inoculum, a proportionality was obtained between the number of RVF virus-infected cells and the inoculum volume. No equivalent relationship was demonstrated when stationary incubation was used with similar volumes of inoculum. The finding of Hodes and Chang[17] that the titer of Shope fibroma virus is independent of the volume of inoculum after stationary incubation and that the efficiency of plating is strongly influenced by the volume of inoculum in animal virus plaque assays[18,19] are further evidence that the practice of using stationary incubation is less efficient for the attachment of virus inoculum onto cell monolayers than the procedure described herein.

Although the rate of RVF virus attachment to cells was markedly similar to that reported for VEE virus,[10] the former was less dependent than the latter on the composition of the menstruum used to obtain maximal attachment of virus. Maximal RVF virus attachment occurred in the presence or absence of calcium and magnesium ions and was unaffected by concentration of serum (Table 2); VEE virus attachment was partially inhibited by these materials.[10] Previous studies indicated that maximal attachment of yellow fever virus was obtained in the presence of medium 199 containing 5% calf serum, with decreasing amounts being attached in PBS containing $MgCl_2$ and $CaCl_2$, and in PBS containing only NaCl.[10] This suggests that the requirements for maximal attachment of different arboviruses to cell monolayers may be highly specific and varied. The rate of RVF virus penetration into cells, however, was comparable to that of VEE virus.[10]

Observations on the development and appearance of fluorescent RVF viral antigens in infected cells agreed with those reported previously.[4,11] The earliest sign of cellular infection was noted in the cytoplasm 5 hours after inoculation; fluorescence was always confined to this site thereafter. Individual infected cells could be counted as early as 12 hours after infection. The appearance of fluorescent foci between 12 and 16 hours after initial infection, however, precluded enumeration of individual infected cells at these times. Fluorescent foci may be prevented by overlaying cell cultures with viral antiserum 1 hour after virus penetration into cells. From observations on the development of cellular infection

by RVF and VEE viruses, the rate of multiplication of these two arboviruses appears to be markedly similar.[10] Of four established cell lines tested for their sensitivity to RVF virus infection, L cells were the most sensitive.

On the basis of comparative virus titrations, the immunofluorescent cell-counting assay of RVF virus was superior in precision, sensitivity, and rapidity to that of the methods of intraperitoneal inoculation of mice and the appearance of cytopathic effects in cell cultures.

LITERATURE CITED

1. Easterday, B.C. 1965. Rift Valley fever. Advances Vet. Sci. 10:65-127.

2. Takemori, N.; Nakano, M.; Hemmi, M. 1955. Plaque formation with Rift Valley fever virus. Virology 1:250-251.

3. Mims, C.A.C. 1956. Rift Valley fever virus in mice: I. General features of the infection. Brit. J. Exp. Pathol. 37:99-109.

4. Iwasa, S. 1959. Multiplication of Rift Valley fever virus in human liver cell culture with special reference to production of complement fixing antigen. Jap. J. Exp. Med. 29:323-334.

5. Matumoto, M.; Saburi, Y.; Nishi, I. 1959. Rift Valley fever virus in the one-day-old chick embryo. J. Immunol. 82:219-225.

6. Runnels, J.L.; Brown, A. 1962. An improved plaque technique for pantropic Rift Valley fever virus. Bacteriol. Proc. p. 159.

7. Easterday, B.C.; Murphy, L.C. 1963. Studies on Rift Valley fever in laboratory animals. Cornell Vet. 53:423-433.

8. Boyle, J.J. 1965. Titration of Rift Valley fever virus in hamster kidney cells in the absence of serum. Amer. J. Vet. Res. 26:190-191.

9. Mahon, N. 1966. Fluorescent cell-counting assay of yellow fever virus. J. Infect. Dis. 116:33-40.

10. Mahon, N.; Cooke, K.O. 1967. Primary virus-cell interactions in the immunofluorescence assay of Venezuelan equine encephalomyelitis virus. J. Virology 1:317-326.

11. Easterday, B.C.; Jaeger, R.F. 1963. The detection of Rift Valley fever virus by a tissue culture fluorescein-labeled antibody method. J. Infect. Dis. 112:1-6.

12. Kaschula, V.R. 1953. The propagation and modification of strains of Rift Valley fever viruses in embryonated eggs and their use as immunizing agents for domestic ruminants. Doctor of Veterinary Science Thesis, University of Pretoria, South Africa.

13. Easterday, B.C.; Murphy, L.C. 1963. The growth of Rift Valley fever virus in cultures of established lines of cells. Cornell Vet. 53:3-11.

14. Riggs, J.L.; Seiwald, R.J.; Burckhalter, J.H.; Downs, C.M.; Metcalf, T.G. 1958. Isothiocyanate compounds as fluorescent labeling agents for immune serum. Amer. J. Pathol. 34:1081-1097.

15. Coons, A.H.; Kaplan, M.H. 1950. Localization of antigen in tissue cells: II. Improvements in a method for the detection of antigen by means of fluorescent antibody. J. Exp. Med. 91:1-13.

16. Rodriguez, J.; Deinhardt, F. 1960. Preparation of a semi-permanent mounting medium for fluorescent antibody studies. Virology 12:316-317.

17. Hodes, M.E.; Chang, L.M.S. 1968. Shope fibroma virus assay based on enumeration of cells containing inclusion bodies. Virology 34: 134-140.

18. Dougherty, R.M. 1964. Animal virus titration techniques, p. 169-223. In R.J.C. Harris (ed.) Techniques in experimental virology. Academic Press, New York.

19. Taniguchi, S.; Yoshino, K. 1964. An analysis of the plaque assay of herpes simplex virus in chick embryo monolayers. Arch. Ges. Virusforsch. 14:537-552.

DOCUMENT CONTROL DATA - R & D

(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)

1. ORIGINATING ACTIVITY (Corporate author)
Department of the Army
Fort Detrick, Frederick, Maryland 21701

2a. REPORT SECURITY CLASSIFICATION
Unclassified

2b. GROUP

3. REPORT TITLE
IMMUNOFLUORESCENT CELL-COUNTING ASSAY OF RIFT VALLEY FEVER VIRUS

4. DESCRIPTIVE NOTES (Type of report and inclusive dates)

5. AUTHOR(S) (First name, middle initial, last name)
Nicholas (NMI) Hahon

6. REPORT DATE	7a. TOTAL NO. OF PAGES	7b. NO. OF REFS
September 1968	23	19

8a. CONTRACT OR GRANT NO.	9a. ORIGINATOR'S REPORT NUMBER(S)
b. PROJECT NO. 1B522301A059	Technical Manuscript 474
c.	9b. OTHER REPORT NO(S) (Any other numbers that may be assigned this report)
d.	

10. DISTRIBUTION STATEMENT
Qualified requesters may obtain copies of this publication from DDC. Foreign announcement and dissemination of this publication by DDC is not authorized. Release or announcement to the public is not authorized.

11. SUPPLEMENTARY NOTES	12. SPONSORING MILITARY ACTIVITY
	Department of the Army Fort Detrick, Frederick, Maryland 21701

13. ABSTRACT

Rift Valley fever virus was quantitatively assayed by enumeration of cells containing fluorescent viral antigens after infection of L cell monolayers. The efficiency and speed of virus attachment were markedly enhanced when augmented by centrifugal force. Approximately 97% of virus inoculum attached to cells within 10 minutes. Thus, a proportionality was obtained between the number of infected cells and volume of inoculum. Virus penetration into cells was linear and complete within 30 minutes at 35 C. From observations on the sequential development of viral antigen within cells and fluorescent cell counts, infected cells were enumerated as early as 12 hours after inoculation of cell monolayers. A linear function was demonstrated between infected cell counts and virus concentration. The immunofluorescent cell-counting assay of Rift Valley fever virus was more precise, sensitive, and rapid than assays based on intraperitoneal inoculation of mice or cytopathic effects in cell cultures.

14. Key Words

*Rift Valley fever virus
*Fluorescent particles
*Assaying
Counting
Cell cultures

DD FORM 1 NOV 68 1473 REPLACES DD FORM 1473, 1 JAN 64, WHICH IS OBSOLETE FOR ARMY USE.